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MECHANISM OF PERCUTANEOUS ABSORPTION. IV. PENETRATION OF NONELECTROLYTES (ALCOHOLS) FROM AQUEOUS SOLUTIONS AND FROM PURE LIQUIDS*

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ABSTRACT

Most quantitative permeability data on human skin are based on studies in which water was used as the vehicle. Very little is known of the permeability of normal stratum corneum as it exists in vivo where it is considerably less hydrated. Similarly very little is known of the effect of nonaqueous solvents on the skin although they are common vehicles in topical therapy.

In this study the permeation rates of a homologous series of primary alcohols (C_1-C_{10}) through skin were measured. The alcohols were applied from aqueous solutions and also as the pure liquids. The permeation behavior in the two cases is compared in terms of (1) the transport rates of the alcohols, (2) the distribution equilibrium between the tissue and vehicle, and (3) the damage to the tissue produced by the vehicle. Fick's law was found to hold as an approximation for both the aqueous and the liquid alcohol systems. Using the measured data, which include permeability and diffusion constants and tissue:vehicle partition coefficients, the diffusional resistance offered by epidermis and dermis is accurately apportioned. The overwhelming role of the stratum corneum in both the aqueous and the pure liquid systems is quantitatively confirmed. The effect of the solubility characteristics of different vehicles on skin permeability is discussed. It is shown that with the aid of a few simple principles and a knowledge of the solubility character of the vehicle, the permeation of different substances can be qualitatively predicted.

It is well accepted that the normal intact stratum corneum is the major rate-limiting barrier to molecular diffusion through human skin. While this tissue is an extremely effective diffusion barrier it is obviously not completely impermeable. Recent studies have aimed at establishing the mechanism of skin permeability [1-4], the role of skin appendages as diffusion shunts [3], the role of solubility [2], and they also have been concerned with explaining the selective permeability of different substances [5, 6]. Much has been learned from these and other studies and the major findings have been reviewed in detail elsewhere [5].

The great majority of this information has been based on measurements made on excised, "intact" human stratum corneum (heat-separated epidermis). Since the permeation of stratum corneum is a passive diffusion process [5], there can be no fundamental difference between in vitro or in vivo permeation as the result of any vital process, e.g., such as active transport. There is ample evidence, for example, which shows that the penetration rate of water through "live" and "dead" skin is the same [5]. However, there can be and there have been significant differences in the experimental conditions which obtain in the typical laboratory measurement of skin permeability in vitro and in the common application of a substance to live skin in a clinical setting.

One of the most important conditions to control and yet the one which has varied the most in the two kinds of experiments is the state of hydration of the stratum corneum. Water has been the most widely used vehicle in quantitative in vitro studies of skin permeability. Therefore most published data relate to well-hydrated, swollen stratum corneum, i.e. tissue after many hours' or days' immersion in water. Extended contact with aqueous solutions is not a common in vivo or clinical experience. More frequently, and particularly with topical therapy, a substance is applied from a nonaqueous vehicle or even a volatile organic solvent. In recent clinical studies appreciable penetration and possible anomalously high penetration was observed when volatile solvents were used [7, †]. But it is difficult to evaluate these results because of our lack of basic information of the permeability of nonhydrated or dehydrated skin. Very little is known of the permeability of "normal" stratum corneum as it exists in vivo where it is considerably less hydrated than when

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† Maibach HI: Private communication with R Scheuplein, San Francisco, 1972

used in vitro. It is known that partial hydration of the stratum corneum by occlusion does increase permeability both in vivo and in vitro. It would seem probable that more complete hydration would have a more profound effect and perhaps would alter the mechanism of permeability.

It is important, therefore, to extend our basic knowledge of skin permeability to more clinically relevant situations.

In an attempt to provide some of this information we have compared the permeability of a series of nonelectrolytes when presented to the skin as aqueous solutions with the same series presented from nonaqueous solvents. The primary alcohols (C_1-C_{10}) were chosen for this study because their permeation from aqueous solutions was reasonably well established and because these alcohols are readily available and relatively inexpensive in isotopically labeled form. We used the liquid alcohols as their own nonaqueous solvents, anticipating that the pure alcohols would not damage the stratum corneum. This expectation proved to be somewhat optimistic.

A further aim of this study was to measure the permeability of epidermis and dermis separately and to apportion to each anatomic layer its share of the diffusional resistance of the whole skin. It has been widely held, but never established (except for water [8]), that the diffusional resistance of the dermis is small in comparison to that of the epidermis. For nonaqueous solvents there are no published data on this matter.

MATERIALS AND METHODS

Details of most of the experimental procedures have been described in earlier publications [1, 2, 4, 6]; references to these are specifically indicated where appropriate in the brief outline given below.

Skin samples. Both epidermal sheets and full-thickness dermis were used in the diffusion experiments. Human, adult abdominal skin obtained at autopsy was used but no further selection was made regarding age or sex. Whole skin was separated by scalpel from the underlying subcutaneous fat. Epidermal sheets (≈100 cm^2) were removed from the dermis after immersion of the whole skin in hot water at 60° C for 30 seconds [2]. The epidermis was floated on water to smooth it out, was taken up on aluminum foil, and stored in the refrigerator at 4° C until used.

Permeability cells. The sheets of dermis or epidermis were supported as diaphragms on standard pyrex diffusion cells. (Photographs and specifications of these cells are given in Ref. 2.) The aqueous alcohol solution or the pure alcohol was placed into the donor side of the cell in contact with the stratum corneum (external) side of the tissue. The receptor side of the cell was filled with distilled water except where indicated and was continuously stirred by a rotating teflon-coated magnet placed inside the cell. The effective diffusion area was 2.54 cm^2 and the receptor volume approximately 2.0 cc [4]. A sampling port in each half of the cell allowed the sampling of both the donor and receptor solutions by microsyringe.

Solubility measurements. Stratum corneum, free of viable epidermal cells [9] was used for determining stratum corneum:water partition coefficients. Approximately 5 mg of tissue were equilibrated with 100 μl of aqueous alcohol solution in a miniature pyrex test tube [6]. Partition coefficients were determined from the loss in concentration of the solution [2]. These partition coefficients correspond to the actual solute (alcohol) distribution between water and stratum corneum. (They are not oil:water coefficients which are occasionally used as approximate estimates to the actual tissue:solvent system.)

Partition coefficients corresponding to the immiscible water:alcohol phases were obtained after thorough shaking of 10 ml of the liquid alcohol with 10 ml of the water containing radioactively labeled alcohol. Measurements of the isotope in both phases were performed as a check on the data.

Analysis of solutions. Two techniques of analysis were used to determine alcohol concentrations: (1) gas-liquid chromatography and (2) radioactive tracer spectrometry. Aqueous alcohol solutions were analyzed mainly by gas chromatography using a column of either Carbowax 1540 or glycerol on Chromasorb-W and a hydrogen flame detector [1]. The permeation rate of water and certain of the alcohols were confirmed by radioactive tracer techniques using a scintillation spectrometer [6].

Representation of data. Permeability constants were computed from the steady state portion of the flux vs time curves by previously discussed methods [2, 4, 5]. A discussion of Fick's law and its application to skin permeability is given in Reference 5.

The most common symbols and their definitions are given in the following list:

List of Symbols and Definitions

k_p . . . Permeability constant for the solute (cm hr^{-1})

K_m . . Partition coefficient = conc. of solute in "tissue"/conc. of solute in vehicle = C_m/C_s

ΔC_s . . Concentration difference of solute across the specified tissue (moles l^{-1})

J_s . . . . Steady state flux of solute (10^{-6} moles cm^{-2} hr^{-1})

D_m . . Calculated (apparent) diffusion constant for the solute and the stratum corneum (cm^2 sec^{-1})

δ_m . . . Solvated thickness of the stratum corneum (microns). Dermal thickness, δ_d, is given in mm.

R . . . . Diffusional resistance (hr cm^{-1}) = $1/k_p$

NOTE:

1. Open and filled circles are used in the figures as data points and are also attached to the symbols as superscripts. The open circles (○) refer to the experiments with the aqueous alcohol solutions; the filled circles (●) refer to the pure liquids (see Fig. 2). Thus k_p° is a permeability constant for an alcohol presented as an aqueous solution and $k_p^{\bullet}$ is a permeability constant for a pure liquid alcohol.

2. Quantities enclosed in brackets are approximated. In most, their direct measurement is extremely difficult to make and such quantities are calculated indirectly from other data using obvious relationships. See Tables I and II.

RESULTS

Effect of Alcohols on the Tissue

Water, organic solvents, and surfactants can either permanently or temporarily alter the effective permeation of substances through the skin, particularly when they are applied in large concentrations. For example, as a barrier medium,

stratum corneum is most effective when dry, less effective when hydrated, and still less effective when solvated with DMSO [10, ‡]. Since in the present study the behavior of the alcohols as solutes was of primary interest and the effect of the solvent on the tissue was secondary, we attempted to maintain a constant tissue:solvent environment insofar as possible. For example, in the series of alcohols presented from aqueous solutions, the stratum corneum was completely hydrated in each case. The small amount of alcohol present in these dilute aqueous solutions did not produce any change in the tissue comparable to the major effect produced by the water. We were able to attribute the observed differences in permeation of the alcohols presented from aqueous solution to the different alcohols per se.

The situation was different for the pure liquid alcohols because they are not equivalent as solvents. Ethanol and methanol in particular alter the stratum corneum to a much greater degree than the other alcohols. Proof of such alteration can be confirmed by permeability experiments in two ways: (1) by showing that the flux produced by a constant solute concentration continuously increases instead of reaching a steady state, or (2) by measuring the permeation of an innocuous solute before and after the tissue has been treated with the suspect solvent and finding that the flux is higher after treatment. The first technique is useful when the suspect solution acts slowly or continuously degrades the tissue, e.g., dilute surfactants [10]. The second method was used for the alcohols because the alteration of the tissue they produce occurs rapidly and afterwards the tissue remains comparatively stable. The data are given in Figure 1. The flux for butanol, the innocuous solute, is 3½ times higher after the tissue was treated with methanol (24-hr continuous contact) and nearly twice as high after ethanol treatment. This method measures only irreversible "damage" to the tissue, since a part of the effect of the solvent in increasing the permeability of the tissue disappears when the solvent is removed and replaced with water. From the uppermost curve in Figure 1, it can be seen that the effective alteration of the tissue in situ with methanol is considerable and that the permeability is increased much more than by a factor of 3½. To a lesser degree ethanol also produced an enhanced permeability; and a very slight enhancement is noticeable with propanol. (See Figure 2.) Liquid butanol itself and the higher alcohols do not appear to cause any permanent alteration of the tissue. This effect of "damage" has been separated out by the device of extrapolating the fluxes back from butanol to water as shown by the dotted line in Figure 2. The fact that the first five alcohols follow a smooth curve when presented as aqueous solutions makes this extrapolation seem plausible.

In all of these experiments the receptor side of the cell, the side in contact with the epidermal cells, was filled with water. This setup was used because it is more akin to the clinical situation where a liquid is applied to the skin surface and penetrates through to the viable epidermis and then through the papillary dermis. Owing to the possible development of strong osmotic forces when two different liquids are separated in this way, we were concerned about possible tissue damage. We tested this possibility by comparing the data from the experiments above with corresponding experiments using the pure (nonisotopic) alcohol as the vehicle on both sides of the tissue. Rather surprisingly we found that the fluxes in the two cases were very much the same. The interpretation of this work is continuing in our laboratory using double-labeling techniques to measure the interchange of each component. But it does seem that the tissue stands up well in spite of the presence of the two different liquids and that the diffusion data obtained are representative and not influenced by osmotic forces.

Permeation of Epidermis

Fluxes. Flux measurements are the primary data of permeability experiments. By themselves, however, flux values are ordinarily not very meaningful, since they vary with the concentrations used and the existing distribution equilibria. We have nevertheless given the fluxes in detail for two reasons: (1) the amount of material passing through the skin under the conditions used is itself of some practical and clinical interest, and (2) it is instructive to see how the permeation of a series of compounds like the alcohols can vary over a very large range and yet be governed by a diffusion constant which itself shows little or no variation.

(Aqueous solutions): Figure 2 depicts the measured fluxes of the alcohols through epidermis as a function of carbon number. Zero carbon number represents water. The lower curve with open circles shows the pattern observed with the aqueous alcohol solutions. The first five alcohols show a moderate and regular increase in flux when penetrating from aqueous solution. Water appears to fit into the pattern next to the smallest and most polar of the alcohols. A maximum in the curve is seen at C_6 above which the fluxes begin to decrease. The fluxes may be seen to vary in a complicated manner throughout the series. The shape of the curve can in part be explained by the fact that different concentrations of the alcohols were used. For example, for the sparingly soluble higher alcohols (C_6–C_{10}) saturated aqueous solutions of decreasing concentration were used; see Table I (ΔC_s°). But the increase in flux for the first five alcohols cannot be explained in this way. In fact the concentrations of the first five alcohol solutions (C_1–C_5) were all identical (0.1 M). In

‡ Scheuplein RJ: "The Permeability of the Skin to Gases." Arlington, Va, Army Res Office, Life Sci Div Ann Rept 1, 1970, p 32.

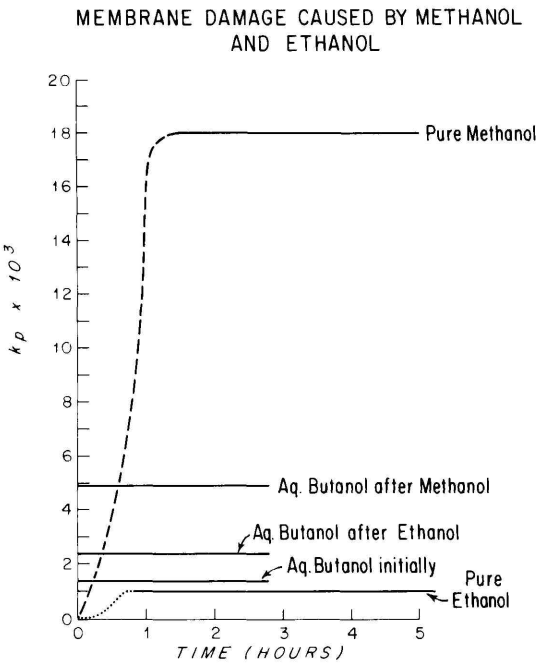

FIG. 1. Damaging effect of methanol and ethanol on the permeability of epidermis. The dotted and dashed curved lines indicate that the permeability is changing over the indicated time interval. These are hypothetical and extrapolated from the measured initial and final steady-state permeability constant (k_p).

order to understand the permeability behavior of these aqueous alcohols other factors must be considered and these will be discussed later.

(Pure liquids): The upper curve in Figure 2 depicts the measured fluxes of the alcohols when applied as pure liquids. As discussed under *Effect of Alcohols on the Tissue*, the shaded portion of this curve represents that portion of the flux attributable to damage produced by the pure alcohol. The dotted line shows the hypothetical flux for the alcohols in the absence of any damage. This adjusted curve for the pure liquids shows the fluxes spanning over 4 orders of magnitude (i.e. over 10,000) while the corresponding concentrations change only fivefold. For the first four alcohols (C_1–C_4) this curve decreases with carbon number in contrast to the curve representing the alcohols applied from aqueous solution. The two curves intersect near C_5, which means that the permeation rates of the higher alcohols (C_5–C_9) are greater when applied as dilute aqueous solution than when applied as the pure liquids. To illustrate a specific case, liquid hexanol (8.2 M) is approximately 150 times more concentrated than saturated aqueous hexanol (0.055 M); yet the permeation rate of aqueous hexanol, far from being 150 times less than the pure liquid, is almost twice as great. This apparently anomalous behavior becomes understandable when the distribution equilibria in each case are considered. See *Fick's Law* (Pure liquids).

Fick's law. (Aqueous solutions): In membrane permeability systems the steady state flux is often directly proportional to the applied concentration. This is Fick's law behavior and it describes quite well the observed permeability of the dilute aqueous alcohol solutions (Ref. 5, p 715). Using Fick's law ($J_s^\circ = k_p^\circ \Delta C_s^\circ$) to obtain permeability constants, and plotting these instead of fluxes against carbon number, the effect of using different concentrations in the experiments can be eliminated. As shown in Figure 3, where both curves are given for comparison, the plot of the permeability constant is less complex than the plot of flux. It thus becomes clear that the maximum at C_6 in the flux curve can be attributed to the experimental artifice of using less concentrated solutions for the higher alcohols; see Table I.

The permeability constant is itself seen to be a function of carbon number, and the relationship can be easily understood in terms of the expression: $k_p^\circ = K_m^\circ D_m^\circ / \delta_m^\circ$. The only parameter in this relation which can account for the 160-fold increase in k_p° is the partition coefficient K_m°. The diffusion coefficient D_m°, if it changes measurably or at all, must decrease with carbon number. This is because the diffusion constant is approximately proportional to $V^{-1/3}$ or $V^{-1/2}$ where V is the molecular volume. From methanol to decanol, V increases by a factor of approximately 4.8. There-

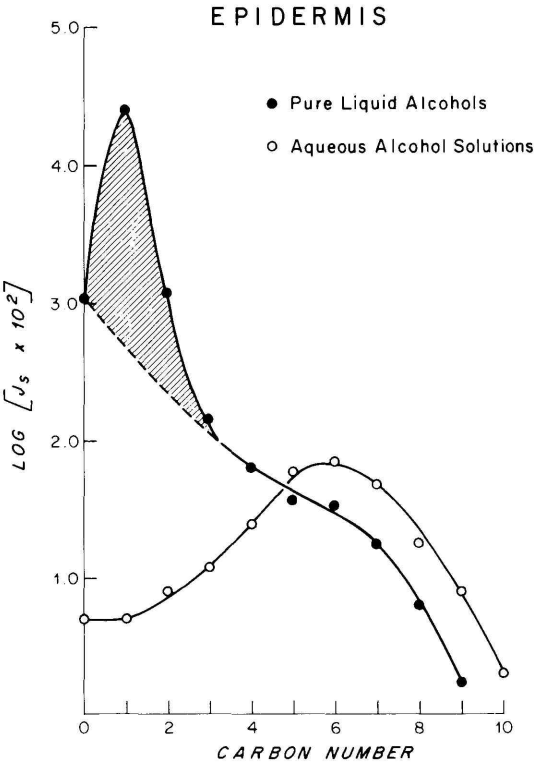

FIG. 2 Permeation rates (J_s) of the alcohols as pure solvents and as solutions through epidermis. Numerical values are tabulated in Tables I and II. The curves cross each other near a value of $J_s = 0.5\ \mu$ moles $cm^{-2}hr^{-1}$.

Table I
PERMEABILITY DATA (25°C) – AQUEOUS ALCOHOL SOLUTIONS (°)

EPIDERMIS

SOLUTE	$k_p^\circ \times 10^3$	ΔC_s°	J_s°	K_m°	$D_m^\circ \times 10^9$	δ_m°	#
WATER	0.5	0.1	0.05	0.88	0.57	[26.6]	45
METHANOL	0.5	0.1	0.05	0.6	0.62	"	10
ETHANOL	0.8	0.1	0.08	0.9	0.66	"	35
PROPANOL	1.2	0.1	0.12	1.1	0.80	"	21
BUTANOL	2.5	0.1	0.25	2.5	0.74	"	8
PENTANOL	6.0	0.1	0.60	5.0	0.88	"	50
HEXANOL	13.0	0.055	0.71	10.0	0.96	"	8
HEPTANOL	32.0	0.015	0.48	30.0	0.79	"	11
OCTANOL	52.0	0.0035	0.18	50.0	0.77	"	13
NONANOL	60.0	0.0014	0.08	—	—	"	3
DECANOL	80.0	0.0003	0.02	—	—	"	1

DERMIS

SOLUTE	$k_p^\circ \times 10^3$	ΔC_s°	J_s°	$[K_d^\circ]$	$D_d^\circ \times 10^6$	δ_d°	#
WATER	60	0.1	6.0	[0.6]	6.9	2.5 mm	10
METHANOL	53	0.1	5.3	"	6.1	"	3
ETHANOL	35	0.1	3.5	"	4.0	"	8
PROPANOL	31	0.1	3.1	"	3.6	"	9
BUTANOL	30	0.1	3.0	"	3.5	"	3
PENTANOL	24	0.1	2.4	"	2.8	"	4
HEXANOL	20	0.055	1.1	"	2.3	"	9
HEPTANOL	25	0.015	0.38	"	2.9	"	9
OCTANOL	26	0.0035	0.09	"	3.0	"	3

(NOTE: the (O) superscript refers to the aqueous system)

k_p° = permeability constant ..... (cm hr^{-1})
ΔC_s° = solute concentration difference ..... (moles l^{-1})
J_s° = flux of solute ..... (μ moles $cm^{-2}\ hr^{-1}$)
K_m° = tissue:solvent partition coefficient of the solute (alcohol) for the aqueous system ..... $C_m^\circ/C_w^\circ = \frac{\text{conc in tissue}}{\text{conc in water}}$
D_m° = apparent membrane diffusion constant for the solute ..... ($cm^2\ sec^{-1}$)
δ_m° = membrane (epidermis or dermis) thickness ..... (microns or mm)
= (The data given are average values; the last column (#) gives the number of experiments on which the given averages are based.)

SAMPLE CALCULATION FOR DERMIS:

$$D_d^\circ(H_2O) = \left(\frac{\delta k_p^\circ}{K_d}\right)^\circ = \frac{0.25 \times 60 \times 10^{-3}}{0.6 \times 3600} = 6.9 \times 10^{-6}\ cm^2\ sec^{-1}$$

fore the diffusion constant D_m° could theoretically decrease by a factor of 2 or 2.2, but no more. The tissue thickness must remain sensibly the same in each experiment since the hydration of the tissue, the primary condition affecting the thickness, is the same in each case. It has been shown that the thickness of stratum corneum increases due to hydration quite slowly, reaching a maximum of 40–50 μ in approximately 3 days [11]. Our estimate for the thickness of the tissue hydrated for 24 hr (the duration of most of the experiments) is $\approx$27 μ which corresponds to 2 gm of water per gm of tissue. Using this value for δ_m° and the experimentally measured partition coefficients K_m° in the relation above, we were able to calculate the diffusion coefficients. These are plotted against carbon number in the lower part of Figure 3. The invariance of D_m° in comparison with k_p° is quite clear and this strongly supports the validity of the analysis.

(Pure liquids): Fick's law cannot be applied, a priori, with the same degree of confidence to the concentrated, pure liquid alcohols. It is a limiting law, i.e. it holds quite well for dilute solutions but is not necessarily true for the higher concentrations. Nonetheless Fick's law behavior may apply in concentrated systems; the test is simply to see whether there is a linear relationship between flux

and concentration throughout the complete range. This was checked for solutions of pentanol in isopropyl myristate, a solvent which permitted pentanol concentrations of 0.01 to 9.0 M. (In these experiments isopropyl myristate was used in both sides of the diffusion chambers.) The permeability of pure pentanol (9.2 M) was also measured (unpublished observations of the authors). A linear relationship between flux and concentration was found throughout the concentration range. We therefore felt justified in using the Fick relation ($k_p^\bullet = J_s/\Delta C_s$) to calculate permeability constants for the pure liquids. The curve showing $k_p^\bullet$ vs "n" is given in Figure 4. In this instance in comparison to the aqueous solutions, moderating the influence of concentration through an application of Fick's law does not greatly simplify the dependence on carbon number. The permeability constant itself appears to decrease over 2 orders of magnitude through the series.

If $k_p^\bullet$ can be expressed by a relation analogous to that applied to the aqueous solutions, e.g., $k_p^\bullet = K_m^\bullet D_m^\bullet/\delta_m^\bullet$ then its decrease must be accounted for by a decrease in this expression. Let us consider each parameter in turn.

$\delta_m^\bullet$: Obviously the thickness of the stratum corneum during contact with different pure alcohols is subject to some change. But this change in thickness must be very slight since the major factor affecting the thickness of the tissue is its state of hydration, and this remained sensibly the same in each case. (Water was always present on the receptor side of the diffusion chamber.) A thickness of the stratum corneum intermediate between 8 μ (the dry average value) and 27 μ (the symmetrically hydrated average value) was assumed to apply in every case. For convenience a specific thickness of 17.7 μ was used. This is a calculated value based on the assumption that a linear water gradient existed within the tissue and an estimate of 2 gm of water per gm of dry tissue for the symmetrically hydrated condition.

$D_m^\bullet$: As we have shown above, the diffusion constant can only decrease with carbon number and then by only a factor of 2-2.2.

$K_m^\bullet$: The partition coefficient, $K_m^\bullet = C_m^\bullet/C^\bullet$, is equal to the ratio of the equilibrium concentration of the alcohol in the tissue (in contact with the liquid alcohol) to the concentration of the liquid alcohol itself. (Activities would be logically more appropriate than concentrations but in our opinion the skin:solvent system does not presently justify this degree of refinement.) The partition coefficient is the only parameter capable of accounting for the observed decrease in $k_p^\bullet$ and to do so it must decrease by over 2 orders of magnitude.

With the techniques at hand, $C_m^\bullet$ could not be directly measured and so an indirect and approximate method to determine $K_m^\bullet$ was used. This method utilized two related equilibria: the stratum corneum:water system (K_m°) and the liquid

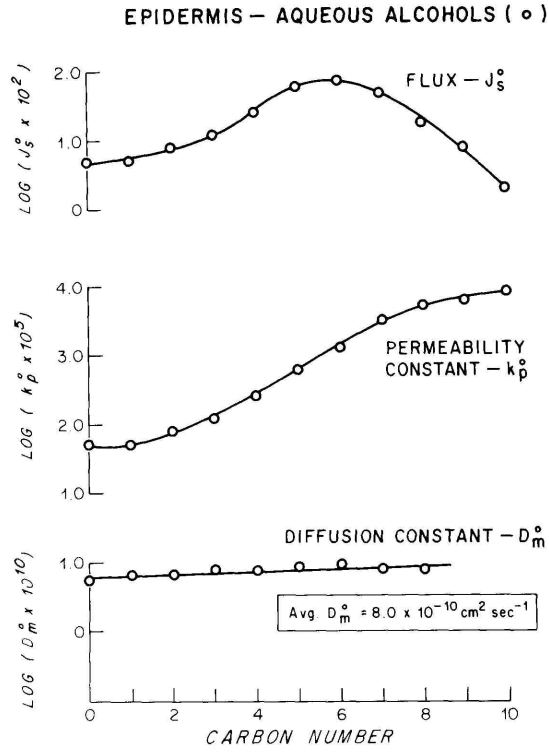

FIG. 3 Permeability data for the aqueous solutions through epidermis as derived from Fick's law. These curves are plotted together to show more clearly the effect of taking into account first, the concentration (ΔC_s° to give k_p° and then, distribution equilibria (i.e. K_m°) and thickness (δ_m°) to yield the diffusion constant (D_m°).

Logarithmic plots are used to include in a single curve the wide range of the data. The exponents are the same for the succeeding three curves in order to facilitate comparison of the fluxes and permeability constants.

alcohol:water system (K). The method was based on the assumed validity of the following expression:

$$K_m^\bullet = \frac{C_m^\bullet}{C^\bullet} \cong \left(\frac{C_m^{\circ}}{C^{\circ}}\right)' \left(\frac{C^{\circ}}{C^\bullet}\right)'' = K_m^\circ \cdot K \qquad \text{[Eq. 1]}$$

This expression assumes that the concentration isotherm for the tissue:water system is linear throughout the concentration range common to both equilibria. This means it is possible to select $C^{\circ\prime}$ equal to $C^{\circ\prime\prime}$ and when this is done C_m° will equal $C_m^\bullet$. The linear condition is met for the higher alcohols with low water solubility and $K_m^\bullet$ was computed as described above for the C_5–C_{10} alcohols. For the water-miscible alcohols K is not defined since the two phases cannot coexist. From vapor sorption studies we known that $K_m^\bullet$ approaches unity for water (actually 0.88 for thoroughly hydrated tissue) and that vapor sorption of C_1, C_2 and C_3 becomes uniformly less. Therefore $K_m^\bullet$ values for these alcohols must lie in order between 0.88 and 0.2 and they have been interpolated in this manner in Table II. Using the

Table II

PERMEABILITY DATA (25°C) - PURE LIQUID ALCOHOLS (•)

EPIDERMIS

SOLUTE	$k_p^\bullet \times 10^3$	$\Delta C_s^\bullet$	$J_s^\bullet$	$[K_m^\bullet]$	$D_m^\bullet \times 10^9$	$[\delta_m^\bullet]$	K	#
WATER	0.2	55.5	11.0	0.44	0.25	[20.0]	—	13
METHANOL	10.4	24.9	259.0	0.3	17.1	[17.7]	—	7
ETHANOL	0.72	17.1	12.3	0.2	1.8	"	—	19
PROPANOL	0.16	13.3	2.12	0.1	0.8	"	—	20
BUTANOL	0.060	10.9	0.65	0.20	0.15	"	0.08	7
PENTANOL	0.051	9.2	0.47	0.10	0.23	"	0.02	9
HEXANOL	0.052	8.2	0.43	0.067	0.38	"	0.0067	4
HEPTANOL	0.025	7.1	0.18	0.063	0.20	"	0.0021	7
OCTANOL	0.010	6.4	0.063	0.028	0.17	"	0.00056	3
NONANOL	0.003	5.8	0.017	—	—	—	0.00024	5
DECANOL	0.0008	5.2	0.0042	—	—	—	—	3

DERMIS

SOLUTE	$k_p^\bullet \times 10^3$	$\Delta C_s^\bullet$	$J_s^\bullet$	$[K_d^\bullet]$	$D_d^\bullet \times 10^6$	$\delta_d^\bullet$	K	#
WATER	60	55.5	3330	0.6	6.9	2.5 mm	—	10
METHANOL	23	24.9	573	0.3	5.3	"	—	2
ETHANOL	7.0	17.1	119	0.2	2.4	"	—	4
PROPANOL	1.8	13.3	24	0.1	1.3	"	—	7
BUTANOL	1.0	10.9	11	0.05	1.4	"	0.08	8
PENTANOL	0.80	9.2	7.3	0.012	4.6	"	0.02	3
HEXANOL	0.35	8.2	2.9	0.004	6.1	"	0.0067	2
HEPTANOL	0.07	7.1	0.5	0.0012	4.0	"	0.0021	9
OCTANOL	0.02	6.3	0.13	0.0003	4.6	"	0.00056	4

(NOTE: the (●) superscript refers to the pure liquid alcohols)
Aside from the superscript the symbols in the table with the exception of K are identical to those in Table I.

K = solubility of the alcohols in water expressed as a distribution coefficient. See text for explanation.

$k_p^\bullet(H_2O)$ = these pure water permeability data are based on water transpiration experiments [12]. The average flux at 25° C reported is 0.2 mg cm^{-2} hr^{-1}. This is converted to a permeability constant and to a diffusion constant by the following calculations:

$$J_s^\bullet(H_2O) = 0.2 \text{ mg cm}^{-2} \text{ hr}^{-1}$$

$$= 11.0 \ \mu \text{ moles cm}^{-2} \text{ hr}^{-1}$$

$$k_p^\bullet(H_2O) = \frac{J_s}{\Delta C_s} = \frac{11.0 \times 10^{-6}}{5.55 \times 10^{-2}} = 0.2 \times 10^{-3} \text{ cm hr}^{-1}$$

$$D_m(H_2O) = \left(\frac{k_p \delta}{K_m}\right)^\bullet = \frac{0.2 \times 10^{-3} \times 20 \times 10^{-4}}{0.44 \times 3600} = 2.53 \times 10^{-10} \text{ cm}^2 \text{ sec}^{-1}$$

values for $K_m^\bullet$ derived from these two semi-empirical methods, the diffusion constants were computed and are plotted in the lower part of Figure 4. Most of the variation through the series has been removed and this lends a measure of support to the approximate methods used in estimating the partition coefficients. The diffusion constant is almost invariant throughout the series and averages 1.3×10^{-10} cm^2 sec^{-1}, about ⅛ of that found for the aqueous system. See Table III.

Permeation Through Dermis

The permeability data from the experiments with full-thickness dermis are developed in a manner identical to that used for epidermis. Tabulated data are recorded in the bottom halves of Tables I and II and the corresponding curves are plotted in Figures 5 and 6.

(Aqueous alcohols): Figure 5 shows that the variation in the flux curve is very well accounted for by eliminating the effect of concentration. The plot of the dermal permeability constant is sensibly invariant except for a slight decrease with carbon number which is also present in the plot of the diffusion constant. This may well be due to the predicted decrease in the diffusion constant due to the increase in molecular volume from C_1-C_8. The main point is that for the dermis the

Table III

AVERAGE DIFFUSION CONSTANTS

TISSUE	D ($cm^2\ sec^{-1}$)
EPIDERMIS (o)	$8.0 \pm 1.1 \times 10^{-10}$
EPIDERMIS (•)	$1.3 \pm 1.4 \times 10^{-10}$
DERMIS (o)	$3.0 \pm 1.4 \times 10^{-6}$
DERMIS (•)	$4.0 \pm 1.5 \times 10^{-6}$

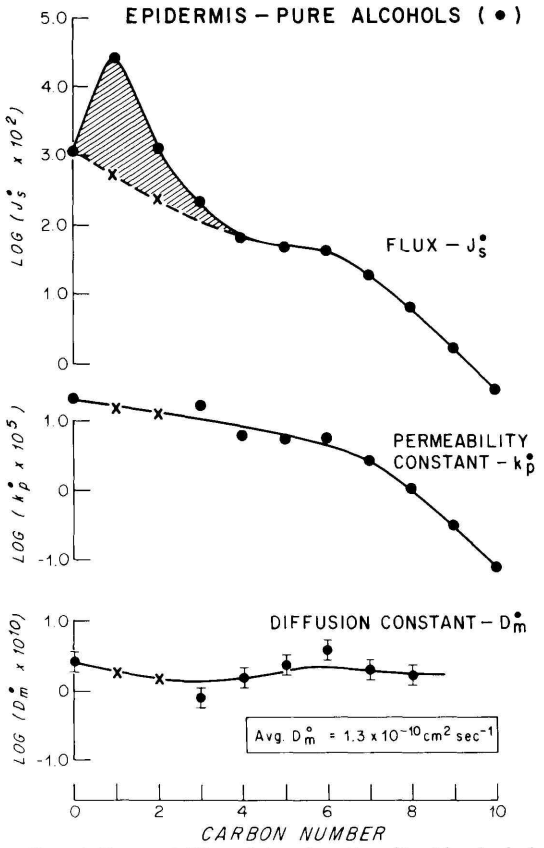

FIG. 4 Permeability data for the liquid alcohols through epidermis as derived from Fick's law. See legend for Fig. 3.

observed variation in flux seems fully attributable to the effect of concentration and no major change in the partition coefficient through the series seems to occur. The average apparent diffusion constant is 3.0×10^{-6} cm^2 sec^{-1} or over 375 times greater than that for epidermis.

(Liquid alcohols): The data for the liquid alcohols are plotted in Figure 6. In contrast to the aqueous alcohols the permeability constant varies over 3 orders of magnitude through the series of pure liquids. It is only after solubility equilibria are taken into account through the partition coefficients that an approximately invariant plot results. These were computed by the same method used for the epidermis (See Eq. 1), i.e. $K_d^\bullet = K_d^\circ K$, except that for dermis, which acts like a watery phase, the K_d°'s were all taken equal to the volume fraction of water in the tissue (0.6). Within experimental error the dermal diffusion constants obtained for the liquid alcohols and for the alcohols presented as aqueous solutions are identical. See Table III.

Apportionment of the Skin's Diffusional Resistance to the Epidermis and Dermis

The skin is a composite diffusion barrier in that penetrating molecules, excepting those entering "shunt" pathways, encounter several structurally different layers in series [5]. The separate diffusional resistances R_i of each layer of a composite diffusion barrier are additive in a manner similar to the electrical resistances in a series electrical circuit. Application of the theory of multibarrier kinetics [5] to diffusion through skin shows that the individual diffusional resistances of the stratum corneum, viable epidermis, and dermis are related to the overall permeability constant by:

$$\frac{1}{k_p} = \sum_{i=1}^{3} R_i = \sum_{i=1}^{3} \left(\frac{\delta}{KD}\right) \qquad \text{[Eq. 2]}$$

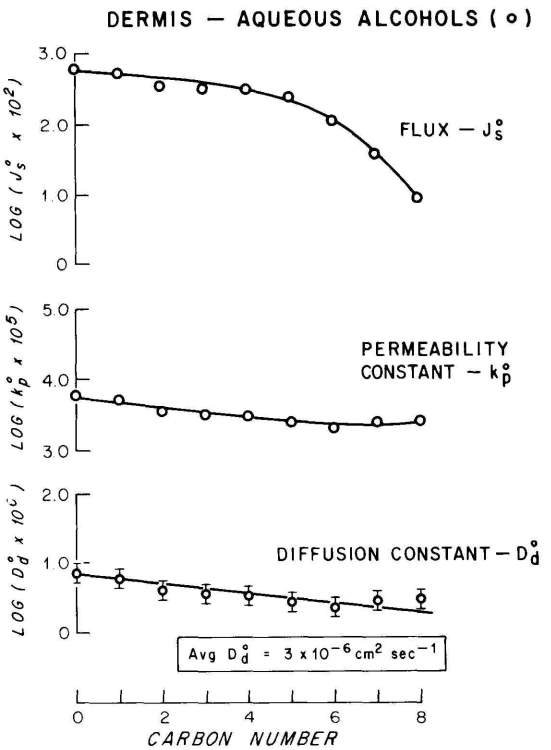

FIG. 5 Permeability data for the aqueous alcohol solutions through dermis as derived from Fick's law. See legend for Fig. 3.

Thus each layer contributes a diffusional resistance equal to the reciprocal of its own separate permeability constant. The data for isolated dermis and epidermis in Tables I, II, and III can be used in this formula to obtain their respective contribution to the diffusional resistance of the whole skin.

For example, using Tables I and II we may compare the effectiveness of hydrated epidermis and dermis as diffusion barriers.

$$R_{skin} = (R_{sc} + R_{v\cdot epi}) + R_d$$
$$= \frac{26.6 \times 10^{-4}\,cm}{8.0 \times 10^{-10}\,cm^2\,sec^{-1}} + \frac{2.5 \times 10^{-1}\,cm}{3.0 \times 10^{-6}\,cm^2\,sec^{-1}} \quad \text{[Eq. 3]}$$
$$= 3.33 \times 10^6 + 8.34 \times 10^4\,cm^{-1}\,sec$$

This calculation ignores distribution effects and thus corresponds to water or to a low-molecular-weight, simple, water-soluble substance with a partition coefficient near unity. It shows that the thin stratum corneum offers fully 40 times more diffusional resistance to the diffusion of water than the full thickness of the dermis. Trypsin digestion of the viable cells of the whole epidermis had no measurable effect on its permeability. This shows that the diffusional resistance of the epidermis is due solely to the stratum corneum.

The diffusional resistances of the alcohols are plotted in Figure 7. The curve for epidermis decreases with "n" because of the increasing values of K which occur in the denominator of expression for R. It should be emphasized that Figure 7 shows the contribution of the full thickness of the dermis, not just the 100–200 μ papillary layer. The curve thus exaggerates what would be the actual resistance in vivo by at least a factor of 10. Even at its full thickness, however, the dermis is for most of the alcohols a far inferior permeability barrier than the stratum corneum. Only for very lipid-soluble alcohols and similar substances is the diffusional resistance of full-thickness dermis comparable to stratum corneum. Table II shows that a similar conclusion holds for the pure liquids.

DISCUSSION

Nature of the Epidermis and Dermis as Diffusion Barriers

The diffusion constants for epidermis and dermis are compared in Table III. These data reveal that the mobility of the alcohol molecules in the dermis is approximately 4 orders of magnitude greater than through the stratum corneum. (The mobility of a molecule is a measure of its average

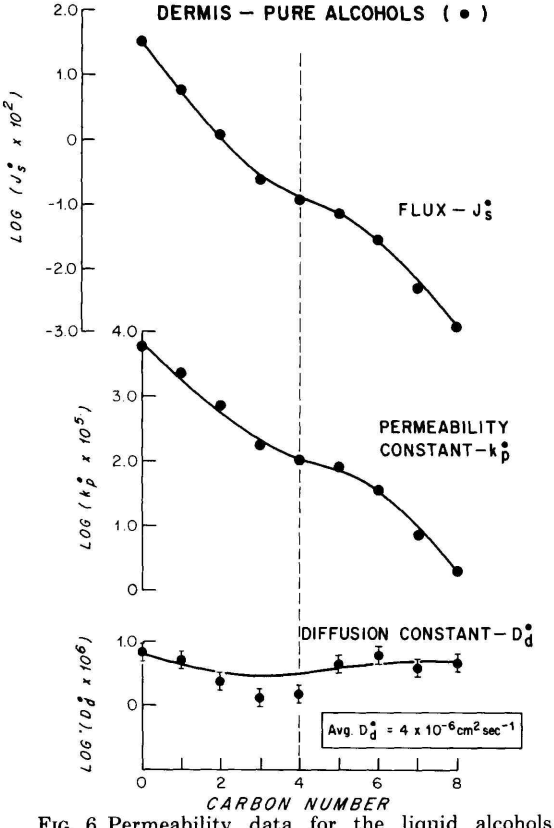

FIG. 6 Permeability data for the liquid alcohols through dermis as derived from Fick's law. See legend for Fig. 3.

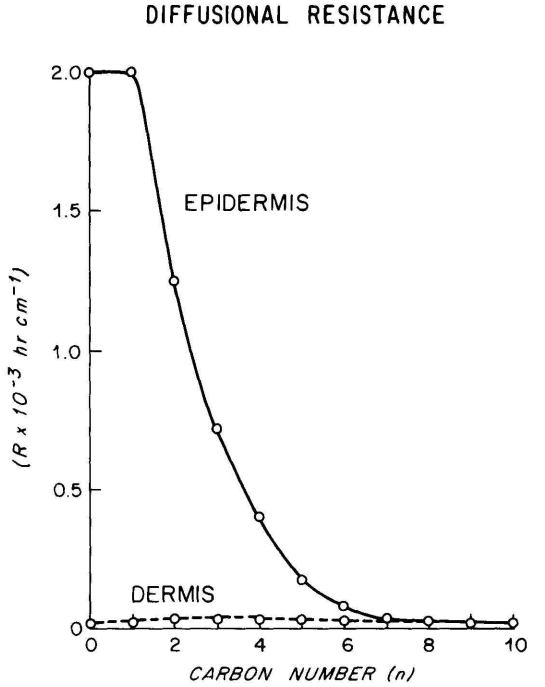

FIG. 7 Comparison of the diffusional resistance (R) of epidermis (stratum corneum) and dermis for the alcohols when presented from water. The lower curve is for full-thickness dermis and thus represents a resistance between 10–20 times greater than the papillary layer which is the significant dermal layer for percutaneous absorption in vivo.

velocity and is proportional to the diffusion coefficient.) Other data [4, 5] show that the activation energy for diffusion through stratum corneum is at least twice that for dermis or viable epidermis (15 vs 6 k cal mol^{-1}). It is evident that the epidermis (i.e. the stratum corneum) is a far more compact tissue than dermis. The partition data (Tables I, II) show that the dermis behaves chemically much like a watery phase. In contrast, the stratum corneum is a more complex medium which possesses both hydrophilic and lipophilic properties. The principal resistance to diffusion appears to be provided by the keratin within the stratum corneum cells. Both electron microscopic evidence and partition data indicate that the keratin is an ultrastructural mozaic of polar (hydrated) regions and intervening lipid regions. This structure endows the tissue with the capacity to discriminate effectively between molecules of different lipid solubility. In contrast, the picture of the dermis which emerges is that of porous, nonselective, watery diffusion medium. Substances very probably diffuse through the solvent-filled interstices of the dermis by a liquid type diffusion mechanism. As reported previously [2, 4, 5] molecules interact much more intimately with the compact stratum corneum and diffusion occurs much more slowly by mechanisms more closely akin to transport processes in solids or fibers.

Effect of Solubility Characteristics of Different Vehicles on Skin Permeability

We have seen that the diffusion constant and the thickness of the tissue both appear to remain constant for the homologous series of penetrating alcohols applied from a specific solvent. It is true that both D and δ for the epidermis are greater for the aqueous system presumably due to the greater hydration and swelling of the stratum corneum, but they remain reasonably constant for all the alcohols penetrating from a given solvent system, i.e. either water or the liquid alcohols (Table III). In the absence of solubility effects, the ratio of these two parameters, D/δ, gives an estimate of the permeability constant as shown by the Fick's law formula. Any change in k_p from this value within the series must originate in solubility phenomena and is accordingly related to the specific K_m or partition coefficient appropriate to the particular solute. For even though D/δ is fixed and therefore the mobility of the molecules and the membrane thickness is fixed, the number of molecules available for diffusion in the tissue immediately adjacent to the vehicle (i.e. concentration in the surface layer) is not fixed. This depends on the distribution of the solute between the vehicle and the tissue and accordingly can vary greatly with different solutes.

The key to understanding the observed trend in k_p in each of the four experimental systems is simply to determine how the distribution equilibrium or K_m changes for each system. The two critical phases are: (1) the vehicle on the donor side of the tissue, and (2) the tissue in contact with this vehicle. Relevant information on the solubility of the alcohols and the solvent nature of the various phases is summarized below. With this information and the solubility principle given above we can readily understand the observed permeability behavior of the alcohols and, in fact, even predict it.

1. Ordering the alcohols with respect to increasing carbon number places them in order of increasing lipid solubility as well as increasing molecular weight.

2. As a solvent medium:
   a. the epidermis is capable of holding in solution both water-soluble and lipid-soluble substances,
   b. the dermis is basically a watery medium,
   c. the liquid alcohols become increasingly lipophilic vehicles as the carbon number increases.

For clarity and convenience we have outlined the four experimental systems below:

Case	Solute	Solvent	Tissue	Trend in k_p as "n" increases	Figure ref.
I	the homologous alcohols	alcohols	stratum corneum	decreasing	4
II	the homologous alcohols	alcohols	dermis	strongly decreasing	6
III	the homologous alcohols	water	dermis	constant	5
IV	the homologous alcohols	water	stratum corneum	increasing	3

In each case we ask the question: How will the increasingly lipid soluble solutes tend to distribute between the two phases?

I: The stratum corneum can accept moderate concentrations of both water-soluble and lipid-soluble substances, but it is not as good a lipid solvent as the alcohols, particularly the higher alcohols. (This may be verified by consulting Tables I and II and comparing K_m° with $1/K$.) The alcohols therefore distribute preferentially in the vehicle phase; this tendency increases with "n" and this will decrease $k_p^{\bullet}$ accordingly. This decrease is shown in Figure 4.

II: The dermis behaves much like water as a solvent medium. It is a far inferior lipid solvent than the stratum corneum. The surface concentration of the alcohols should therefore decrease more rapidly than in case I and lower the $k_p^{\bullet}$ even

more. This is confirmed in Figure 6; $k_p^{\bullet}$ decreases approximately 3.5 orders of magnitude as compared with roughly 2 orders of magnitude for Figure 4.

III: Both phases, the solvent (water) and the tissue (dermis), are watery media. The alcohols can have no strong preference for either phase and therefore k_p° does not change in a major way through the series (Fig. 5).

IV: The solvent (water) is an inferior lipid solvent compared to the tissue (stratum corneum) particularly for the higher alcohols. Therefore as "n" increases, a higher solute concentration in the tissue will develop and k_p° will increase. Because of the capacity of both water and the tissue to dissolve the lower alcohols, k_p° increases slowly at first since there is no marked preference. While each additional methylene unit increases the lipid character of the solute, the effectiveness of additional carbons decreases after C_8 and the increase in k_p° should eventually diminish. These two effects produce the S-shaped curve as shown in Figure 3.

Thus the general decreasing tendency of the curves for both cases I and II arise from fundamentally the same cause; the tissue phase is not as good a lipid solvent as the vehicle. In case IV just the opposite is true.

These experiments show that whether substances are applied to the skin from water or from a nondeleterious organic solvent we can anticipate Fick's law to be obeyed. With a knowledge of the solubility of the solute in the vehicle and in the tissue we are in a position to predict the rate of percutaneous absorption.

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